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DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L13	L12 and (captur$3 or bind$3)	7	L13
L12	(nucleic acid or nucleotide sequence$1) near5 encode$1 near5 interact$3 near5 domain$1	9	L12
L11	l8 and ((nucleic acid or nucleotide) near5 (captur$3 or bind$3))	66	L11
L10	l8 and (captur$3 or bind$3)	127	L10
L9	L8 and (moiety captur$3 or moiety bind$3)	1	L9
L8	(nucleic acid or nucleotide sequence$1) near5 encode$1 near5 interact$3	153	L8
L7	L5 AND (INTERACT$3 NEAR5 DOMAIN)	0	L7
L6	L5 and (encode$1 near5 interact$3)	0	L6
L5	(nucleotide sequence$1 or nucleic acid sequence$1) near5 moiety bind$3	2	L5
L4	L3 and non complementary primer$1	2	L4
L3	(detect$3 or determin$3) near5 contaminat$3	10164	L3
L2	L1 and (non-complementary near5 primer$1)	13	L2
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L1	(cross contaminat$3) near5 amplif$7	99	L1

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☑ 1. 6528634 . 17 Oct 96; 04 Mar 03. Aiolos gene. Georgopoulos; Katia, et al. 536/23.5; 435/320.1 435/325 435/69.1. C07H021/04.

☑ 2. 6479653 . 13 Jul 00; 12 Nov 02. Compositions and method for regulation of transcription. Natesan; Sridaran, et al. 536/23.4; 435/320.1 435/325. C07H021/04.

☑ 3. 6117680 . 26 Aug 98; 12 Sep 00. Compositions and methods for regulation of transcription. Natesan; Sridaran, et al. 435/455; 435/235.1 435/320.1 435/325 435/456 536/23.4. C12N005/10 C12N015/63.

☑ 4. 6020143 . 03 Jul 97; 01 Feb 00. Method for identifying substances that affect the interaction of a presenilin-1-interacting protein with a mammalian presenilin-1 protein. St. George-Hyslop; Peter H., et al. 435/7.1; 530/350. C12Q001/00 C07K014/00.

☑ 5. 5858711 . 25 Nov 96; 12 Jan 99. NF-AT-interacting protein NIP45 and methods of use therefor. Glimcher; Laurie H., et al. 435/69.1; 435/29 435/320.1 435/325 536/23.5 536/24.5. C07H021/04 C12N015/00 C12P021/06 C12Q001/02.

☐ 6. WO 200292015 A2 . Regulating LRP5, LRP6 or HBM activity in a subject, useful for modulating lipid levels and/or bone mass, and for in treating bone mass disorders, e.g. osteoporosis, comprises administering a composition which modulates a Dkk activity. ALLEN, K, et al. A61K000/00.

☐ 7. US 20030091999 A1 WO 200125249 A1 AU 200077211 A US 20010046680 A1 EP 1226149 A1 . Novel nucleic acid molecule comprising a sequence that has a group binding region (GBR) and encodes an interacting domain which directly or indirectly binds with the GBR. YU, Z. A61K038/00 C07H021/02 C07H021/04 C07K014/00 C12Q001/68 C12Q001/70 G01N033/53.

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L13: Entry 5 of 7 File: USPT Jan 12, 1999

DOCUMENT-IDENTIFIER: US 5858711 A
TITLE: NF-AT-interacting protein NIP45 and methods of use therefor

Brief Summary Text (7):
While the molecular basis for the tissue-specific expression of T cell cytokines has remained elusive, study of the transcriptional elements of cytokine genes has provided insight into their regulation. Analysis of the IL-4 cytokine promoter, for example, has revealed functionally critical sites for several transcription factors including members of the NF-AT and AP-1 families (Rooney, J. W. et al. (1995) Immunity 2:473-483; Szabo, S. J. et al. (1993) Mol. Cell. Biol. 13:4793-4805). NF-AT is a multisubunit transcription complex that contains a cyclosporin A sensitive cytoplasmic phosphoprotein and an inducible nuclear component composed of AP-1 family member proteins (Flanagan, W. M. et al. ((1991) Nature 352:803-807; Jain, J. et al. (1992) Nature 356:801-804). Purification and cloning of NF-ATp revealed a region of limited sequence identity to the Rel Homology Domain (RHD) of the NF.kappa.B family of transcription factors (McCaffrey, P. G. et al. (1993) Science 262:750-754). Subsequent cloning and sequencing of three related genes, NF-ATc, NF-AT4/x/c3, and NF-AT3/c4 revealed similar domains. NF-AT family members share approximately 70% sequence similarity within this domain and approximately 18% sequence similarity to the RHD of the Rel/NF.kappa.B family of transcription factors. Consistent with their very limited sequence similarity in the RHD, there are marked differences in the behavior of NF.kappa.B and NF-AT proteins, and much less is known about the pathways that mediate transcriptional regulation of NF-AT target genes. However, considering that NF-AT family members can bind to and transactivate the promoters of multiple cytokine genes including IL-2 and IL-4 (Rooney, J. et al. (1995) Immunity 2:545-553; Szabo, S. J. et al. (1993) Mol. Cell. Biol. 13:4793-4805; Flanagan, W. M. et al. (1991) Nature 352:803-807; Northrop, J. P. et al. (1994) Nature 369:497), NF-AT proteins are not likely to be directly responsible for mediating Th1- or Th2-specific cytokine transcription.

Brief Summary Text (8):
Most, if not all, NF-AT binding sites in cytokine promoter regulatory regions are accompanied by nearby sites that bind auxiliary transcription factors, usually members of the AP-1 family. It has been shown that NF-AT and AP-1 proteins bind coordinately and cooperatively and are required for full activity of the IL-2 and IL-4 promoters. Different AP-1 proteins, specifically c-Jun, c-Fos, Fra-1, Fra-2, Jun B and Jun D, have been shown to bind to these sites (Rao, A. et al. (1994) Immunol. Today 15:274-281; Jain, J. et al. (1993) Nature 365:352-355; Boise, L. H. et al. (1993) Mol. Cell. Biol. 13:1911-1919; Rooney, J. et al. (1995) Immunity 2:545-553; Rooney, J. et al. (1995) Mol. Cell Biol. 15:6299-6310). However, none of these AP-1 proteins is expressed in a Th1- or Th2-specific manner and there is no evidence for the differential recruitment of AP-1 family members to the IL-2 or IL-4 composite sites (Rooney, J. et al. (1995) Mol. Cell. Biol. 15:6299-63 10). Thus, neither NF-AT proteins nor the AP-1 family members c-Jun, c-Fos, Fra-1, Fra-2, Jun B and Jun D can account for the tissue-specific transcription of IL-4 in Th2 cells.

Brief Summary Text (15):
The NIP45 proteins of the invention, or fragments thereof, can be used to prepare anti-NIP45 antibodies. Accordingly, the invention further provides an antibody that specifically binds NIP45 protein. In one embodiment, the antibody is monoclonal. In another embodiment, the antibody is labeled with a detectable substance.

Drawing Description Text (14):
FIG. 8 is a photograph of CAT assay results (left) and a bar graph quantitating the relative fold induction of CAT activity (right) in HepG2 cells transfected with a 3.times. NF-AT-CAT reporter gene construct (containing three NF-AT binding sites) and

either a control expression plasmid or an NF-AT family expression plasmid (NF-ATp, NF-ATc, NF-AT3 or NF-AT4), alone (-) or in combination with a NIP45 expression plasmid (+).

Detailed Description Text (2):
This invention pertains to NF-AT Interacting Protein 45 (NIP45), a 45 kDa protein that interacts with NF-AT proteins. A cDNA encoding NIP45 was isolated based upon the interaction of NIP45 with the RHD of NF-ATp using a two-hybrid interaction trap assay in yeast (see Example 1). Coimmunoprecipitation experiments demonstrated that NIP45 and NF-AT interact in vivo in mammalian cells (see Example 2). The cDNA encoding NIP45 has been sequenced and characterized (see Example 3). Examination of the tissue expression pattern of NIP45 mRNA revealed that the NIP45 transcript is preferentially expressed in spleen, thymus and testis (see Example 4). Subcellular localization studies demonstrated that NIP45 protein is evenly distributed throughout the cell nucleus (see Example 5). Functional studies showed that NIP45 synergizes with NF-AT to stimulate transcription from promoters containing NF-AT binding sites and, moreover, synergizes with NF-AT and c-Maf to stimulate transcription from the IL-4 promoter (see Example 6). Moreover, NIP45, NF-AT and c-Maf can act in concert to induce expression of the endogenous IL-4 gene in cells that do not normally express IL-4 (e.g, B cells.) (see Example 7).

Detailed Description Text (17):
As used herein, the term "antibody" is intended to include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as Fab and F(ab').sub.2 fragments. The terms "monoclonal antibody" and "monoclonal antibody composition", as used herein, refer to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of an antigen. A monoclonal antibody composition thus typically displays a single binding affinity for a particular antigen with which it immunoreacts.

Detailed Description Text (28):
An isolated nucleic acid molecule encoding a NIP45 protein homologous to the protein of SEQ ID NO: 2 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO: 1 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO: 1 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, an amino acid residue in NIP45 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a NIP45 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for their ability to interact with an NF-AT RHD (e.g, using a GST-NF-AT-RHD fusion protein) to identify mutants that retain NF-AT-interacting ability. Following mutagenesis of SEQ ID NO: 1, the encoded mutant protein can be expressed recombinantly in a host cell and the ability of the mutant protein to interact with NF-AT can be determined using an in vitro interaction assay. For example, a recombinant NIP45 protein (e.g., a mutated or truncated form of SEQ ID NO: 2) can be radiolabeled and incubated with a GST-NF-AT RHD fusion protein. Glutathione-sepharose beads are then added to the mixture to precipitate the NIP45-GST-NF-AT RHD complex, if such a complex is formed. After washing the beads to remove non-specific binding, the amount of radioactive protein associated with the beads is determined and compared to the amount of radioactive protein remaining in the eluate to thereby determine whether the NIP45 protein is capable of interacting with the RHD of NF-AT.

Detailed Description Text (36):
Expression of proteins in prokaryotes is most often carried out in E. coli with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D. B. and Johnson, K. S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Detailed Description Text (56):
An isolated NIP45 protein, or fragment thereof, can be used as an immunogen to generate antibodies that bind NIP45 using standard techniques for polyclonal and monoclonal antibody preparation. The NIP45 protein can be used to generate antibodies or, alternatively, an antigenic peptide fragment of NIP45 can be used as the immunogen. An antigenic peptide fragment of NIP45 typically comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO: 2 and encompasses an epitope of NIP45 such that an antibody raised against the peptide forms a specific immune complex with NIP45. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of NIP45 that are located on the surface of the protein, e.g, hydrophilic regions. A hydrophobicity analysis of the NIP45 protein sequence of SEQ ID NO: 2 is shown in FIG. 5.

Detailed Description Text (58):
Accordingly, another aspect of the invention pertains to anti-NIP45 antibodies. Polyclonal anti-NIP45 antibodies can be prepared as described above by immunizing a suitable subject with a NIP45 immunogen. The anti-NIP45 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized NIP45. If desired, the antibody molecules directed against NIP45 can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-NIP45 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256:495-497) (see also, Brown et al. (1981) J. Immunol 127:539-46; Brown et al. (1980) J Biol Chem 255:4980-83; Yeh et al. (1976) PNAS 76:2927-31; and Yeh et al. (1982) Int. J. Cancer 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al. (1983) Immunol Today 4:72), the EBV-hybridoma technique (Cole et al. (1985), Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in Monoclonal Antibodies: A New Dimension In Biological Analyses, Plenum Publishing Corp., New York, N.Y. (1980); E. A. Lerner (1981) Yale J. Biol. Med., 54:387-402; M. L. Gefter et al. (1977) Somatic Cell Genet., 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a NIP45 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds NIP45.

Detailed Description Text (59):
Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-NIP45 monoclonal antibody

(see, e.g., G. Galfre et al. (1977) Nature 266:55052; Gefter et al. Somatic Cell Genet., cited supra; Lerner, Yale J. Biol. Med., cited supra; Kenneth, Monoclonal Antibodies, cited supra). Moreover, the ordinary skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines may be used as a fusion partner according to standard techniques, e.g, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from the American Type Culture Collection (ATCC), Rockville, Md. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind NIP45, e.g, using a standard ELISA assay.

Detailed Description Text (60):
Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-NIP45 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with NIP45 to thereby isolate immunoglobulin library members that bind NIP45. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP.TM. Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner et al. U.S. Pat. No. 5,223,409; Kang et al. International Publication No. WO 92/18619; Dower et al. International Publication No. WO 91/17271; Winter et al. International Publication WO 92/20791; Markland et al. International Publication No. WO 92/15679; Breitling et al. International Publication WO 93/01288; McCafferty et al. International Publication No. WO 92/01047; Garrard et al. International Publication No. WO 92/09690; Ladner et al. International Publication No. WO 90/02809; Fuchs et al. (1991) Bio/Technology 9:1370-1372; Hay et al. (1992) Hum Antibod Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffiths et al. (1993) EMBO J 12:725-734; Hawkins et al. (1992) J Mol Biol 226:889-896; Clarkson et al. (1991) Nature 352:624-628; Gram et al. (1992) PNAS 89:3576-3580; Garrad et al. (1991) Bio/Technology 9:1373-1377; Hoogenboom et al. (1991) Nuc Acid Res 19:4133-4137; Barbas et al. (1991) PNAS 88:7978-7982; and McCafferty et al. Nature (1990) 348:552-554.

Detailed Description Text (68):
Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

Detailed Description Text (71):
Another aspect of the invention pertains to a method of using the various NIP45 compositions of the invention. For example, the invention provides a method for detecting the presence of NIP45 protein or mRNA in a biological sample. The method involves contacting the biological sample with an agent capable of detecting NIP45 protein or mRNA such that the presence of NIP45 protein or mRNA is detected in the

biological sample. A preferred agent for detecting NIP45 mRNA is a labeled nucleic acid probe capable of hybridizing to NIP45 mRNA. The nucleic acid probe can be, for example, the NIP45 cDNA of SEQ ID NO: 1, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 nucleotides in length and sufficient to specifically hybridize under stringent conditions to NIP45 mRNA. A preferred agent for detecting NIP45 protein is a labeled antibody capable of binding to NIP45 protein. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab').sub.2) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids. For example, techniques for detection of NIP45 mRNA include Northern hybridizations and in situ hybridizations. Techniques for detection of NIP45 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence.

Detailed Description Text (79):
Isolated NIP45 and/or NF-AT family proteins may be used in the method, or, alternatively, only portions of NIP45 and/or an NF-AT family protein may be used. For example, an isolated NF-AT Rel Homology Domain (or a larger subregion of NF-AT that includes the RHD) can be used as the NIP45-interacting portion of NF-AT. Likewise, a portion of NIP45 capable of binding to the NF-AT RHD may be used. In a preferred embodiment, one or both of (i) and (ii) are fusion proteins, such as GST fusion proteins (e.g., GST-NF-AT RHD can be used as the NIP45-interacting portion of NF-AT). The degree of interaction between (i) and (ii) can be determined, for example, by labeling one of the proteins with a detectable substance (e.g., a radiolabel), isolating the non-labeled protein and quantitating the amount of detectable substance that has become associated with the non-labeled protein. The assay can be used to identify agents that either stimulate or inhibit the interaction between NIP45 and an NF-AT family protein. An agent that stimulates the interaction between NIP45 and an NF-AT family protein is identified based upon its ability to increase the degree of interaction between (i) and (ii) as compared to the degree of interaction in the absence of the agent, whereas an agent that inhibits the interaction between NIP45 and an NF-AT family protein is identified based upon its ability to decrease the degree of interaction between (i) and (ii) as compared to the degree of interaction in the absence of the agent. Assays systems for identifying agents that modulate SH2 domain-ligand interactions as described in U.S. Pat. No. 5,352,660 by Pawson can be adapted to identifying agents that modulate the NIP45/NF-AT RHD interaction.

Detailed Description Text (101):
The method of the invention for identifying proteins that interact with NIP45 can be designed based on the two-hybrid assay system (also referred to as an interaction trap assay) known in the art (see e.g, Field U.S. Pat. No. 5,283,173; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Biotechniques 14:920-924; and Iwabuchi et al. (1993) Oncogene 8:1693-1696). The two-hybrid assay is generally used for identifying proteins that interact with a particular target protein. The assay employs gene fusions to identify proteins capable of interacting to reconstitute a functional transcriptional activator. The transcriptional activator consists of a DNA-binding domain and a transcriptional activation domain, wherein both domains are required to activate transcription of genes downstream from a target sequence (such as an upstream activator sequence (UAS) for GAL4). DNA sequences encoding a target "bait" protein are fused to either of these domains and a library of DNA sequences is fused to the other domain. "Fish" fusion proteins (generated from the fusion library) capable of binding to the target-fusion protein (e.g., a target GAL4-fusion "bait") will generally bring the two domains (DNA-binding domain and transcriptional activation domain) into close enough proximity to activate the transcription of a reporter gene inserted downstream from the target sequence. Thus, the "fish" proteins can be identified by their ability to reconstitute a functional transcriptional activator (e.g., a functional GAL4 transactivator).

Detailed Description Text (102):
This general two-hybrid system can be applied to the identification of proteins that interact with NIP45 by construction of a target NIP45 fusion protein (e.g., a NIP45/GAL4 binding domain fusion as the "bait") and a cDNA library of "fish" fusion proteins (e.g., a cDNA/GAL4 activation domain library). The cDNA library can be prepared from a cell type of interest to identify proteins in that cell type that interact with NIP45. For example, the cDNA library can be prepared from T cells to identify proteins in T cells that interact with NIP45. The expression vector encoding the NIP45 fusion protein and the cDNA library are then introduced into a host cell that also contains a reporter gene construct linked to a regulatory sequence responsive to NIP45 (e.g, a region of the IL-4 promoter or a promoter containing NF-AT sites). cDNAs encoding proteins that interact with NIP45 can be identified based upon transactivation of the reporter gene construct. For further description of the two hybrid assay system, see Example 1.

Detailed Description Text (107):
A yeast two-hybrid interaction trap assay was used to isolate proteins that could directly bind to the RHD of NF-ATp. An NF-ATp(RHD)-Gal4 fusion protein was prepared for use as the "bait" in the yeast two-hybrid assay by cloning a 900 bp fragment of murine NF-ATp (McCaffrey, P. G. et al. (1993) Science 262:750-754), spanning amino acids 228 to 520, into the BamHI site of vector PEG202 (Gyuris, J. et al. (1993) Cell 75:791-803). In frame fusion of the NF-AT(p) polypeptide sequences to the Gal4 sequences was confirmed by DNA sequence analysis. This bait was used to screen a cDNA library prepared from the murine T cell line D10, constructed in the plasmid pJG4-5, to select for clones encoding polypeptides that interacted with the bait, using methodologies known in the art (see Gyuris, J. et al. (1993) Cell 75:791-803).

Detailed Description Text (131):
Although a reporter construct containing multiple copies of the NF-AT binding site provides a sensitive method for measuring transactivation by NF-AT and NIP45, we sought to determine if NIP45 was functional in the context of a native NF-AT-dependent promoter. IL-4 expression is highly tissue specific and restricted to the Th2 subset of T cells and to mast cells. The IL-4 promoter contains multiple NF-AT binding sites which have been shown to be critical for expression of IL-4 (Rooney, J. W. et al. (1995) Immunity 2:473-483). Furthermore, the proto-oncogcne c-maf has been shown to direct tissue specific expression of IL-4 (U.S. Ser. No. 08/636,602). Thus, the IL-4 promoter is not active in the HepG2 cell line but can be activated by the introduction of NF-ATp and c-maf. In cotransfection experiments carried out as described above, HepG2 cells were transfected with an IL-4-CAT reporter construct (extending to -732 bp of the IL-4 promoter) and expression vectors or controls for NIP45, NF-ATp and c-Maf. The controls for NIP45 was a frame shift mutant at amino acid 13. Controls for NF-ATp and c-Maf were the empty expression vectors pREP4 and pMEX respectively (Ho, I. C. et al. (1996) Cell 85:973-983). The results of these experiments are shown in FIG. 9 (representative CAT assays and bar graphs are depicted as in FIG. 8). The data indicate that introduction of NIP45 together with NF-ATp and c-Maf results in an additional 9-fold increase in the activity of the IL-4 promoter relative to that seen for NF-ATp and c-Maf alone. NIP45 also increased the activity of the IL-4 promoter in the absence of transfected NF-ATp, an effect likely due to interaction with endogenous NF-ATp.

Other Reference Publication (14):
Hodge, M.R. et al., "The proximal promoter of the IL-4 gene is composed of multiple essential regulatory sites that bind at least two distinct factors", Journal of Immunology vol. 154, No. 12, pp. 6397-6405 (1995).

CLAIMS:

35. An isolated nucleic acid molecule comprising a nucleotide sequence at least 60% homologous to the nucleotide sequence of SEQ ID NO: 1, wherein the nucleic acid molecule encodes a protein that interacts with the Rel Homology Domain of an NF-AT family protein.